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Date of Deposit: November 20, 2003

Selena Whitaker-Paquiet

Attorney Docket No. 11000.1037c3
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	**Matthew Sleeman, Nevin Abernethy, and James G. Murison (as amended 06/23/03)**
		Group Art Unit: 1646
Application No.	:	09/823,038
Filed	:	March 28, 2001
For	:	**COMPOSITIONS ISOLATED FROM STROMAL CELLS AND METHODS FOR THEIR USE**
Examiner	:	Ruixiang Li

DECLARATION OF DR. J. GREG MURISON

MS: NON-FEE AMENDMENT
Commissioner for Patents
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Alexandria, VA 22313-1450

The undersigned, Dr. J. Greg Murison, hereby declares:

1. I am presently a Senior Staff Scientist at Genesis Research and Development Corporation Limited, the assignee of the subject patent application, and an inventor of the claimed subject matter. I have a PhD in the field of Immunology. The following studies were carried out under my supervision.

2. The effects of administration of FGFR5 protein *in vivo* were examined as follows:

Experiments 1 and 2

Experiment 1 used BALB/cByJ mice and experiment 2 used C3H/HeJ mice. Both sets of mice were injected subcutaneously with 5 μg (55 nM in 0.1 ml PBS) of murine FGFR5β extracellular domain (ECD; amino acids 22 - 373 of SEQ ID NO: 31) - murine IgG3 Fc fusion protein (prepared as described in Example 5 of the instant specification) in the morning and the same dose in the evening (i.e. each mouse received 10 μg per day) for five days. Control mice received PBS alone. On the sixth day, the mice were sacrificed and the draining lymph nodes (axillary and lateral axillary) were removed. A single cell suspension was generated from the lymph nodes of each mouse and the number of cells collected from each mouse was determined by trypan blue viability counting using a haemocytometer. The lymph node cells collected from the FGFR5-treated mice were then pooled. The lymph node cells collected from the PBS-treated mice were amalgamated into a separate pool of cells. The cells from both the FGFR5 and PBS-treated mice were then stained for the cell surface antigens listed in Table 1, below, and analyzed by flow cytometry. The data provided in Table 1 is expressed as a percentage of the lymph node cells that express the marker examined.

Experiment 3

In this experiment, C3H/HeJ mice were injected subcutaneously with 10 μg (110 nM in 0.1 ml PBS) of murine FGFR5β ECD - human IgG1 Fc fusion protein in one injection per day for 5 days. While the treatment regime differed from that used in Experiments 1 and 2 above, the total dose of protein administered to the mice did not alter. Control mice were administered human IgG1 Fc fragments alone. On the sixth day, the mice were sacrificed and the draining lymph nodes (axillary and lateral axillary) removed. The number of cells collected from each mouse and the presence of cell surface antigens was determined as described above.

In all three of these experiments, *in vivo* administration of FGFR5 stimulated lymphadenopathy, or enlargement of the lymph nodes. More specifically, administration of FGFR5 was found to result in a preferential increase in the frequency of B cells in the

draining lymph nodes. When compared to mice treated with Fc protein, the frequency of B cells doubled in the draining lymph nodes of FGFR5-treated mice. An analysis of the cell cycle state of the B cells by flow cytometry indicated that they were not expanding but were either selectively migrating or being retained in the lymph nodes. The cells are, however, activated as there is an increase in the number of cells expressing the very early activation antigen, CD69. Similar results were obtained for both the murine FGFR5β ECD - human IgG1 Fc fusion protein and the murine FGFR5β ECD - murine IgG3 Fc fusion protein, demonstrating that the effects are caused by the segment common to both fusion proteins, i.e. the murine FGFR5β extracellular domain.

Table 1: Comparison of three *in vivo* experiments testing the effects of *in vivo* administration of soluble FGFR5 in mice

(The values in this table represent the percentage of total lymph node cells expressing the indicated marker)

		Experiment 1		Experiment 2		Experiment 3	
		Balb/c		C3H/HeJ		C3H/HeJ	
Markers	Cell type recognized	Murine Fc FGFR5	PBS	Murine Fc FGFR5	PBS	Human Fc FGFR5	Human Fc
CD3	T cell	63	81	59	82	32	67
CD19	B cell	35	21	39	16	61	26
Class II	B cell and macrophage	41	20	ND*	ND	ND	ND
CD45R	B cell	ND	ND	ND	ND	72	31
CD69	Activated cells	23	14	18	10	21	10

* ND = Not determined

Experiment 4

The popliteal lymph node assay was used to assess the effects of treating mice with a murine FGFR5γ-Fc fusion protein. The polypeptide sequence for FGFR5γ is provided in SEQ ID NO: 32. Groups of 4 BALB/cByJ mice were injected with 50μg of FGFR5γ-Fc under the left hind footpad and 50μg of the control protein FGFR2-Fc under the right

hind footpad. In addition, groups of two mice were injected with PBS under the left hind footpad to compare the effects of FGFR5, FGFR2 and PBS. The lymphatics from this site drain to the popliteal lymph node. These lymph nodes were collected 1, 2 and 3 days after the initiation of the experiment and the cells from each node were released and counted using a haemocytometer, and their viability assessed by the Trypan blue exclusion assay. The cells from the individual nodes were then stained with fluorescently labeled antibodies and the relative frequencies of each of the major haemopoietic cell types assessed by flow cytometry.

The results of these assays are shown in Figs. 1-5 submitted herewith. Specifically, Fig. 1 shows that subcutaneous administration of FGFR5γ-Fc was found to induce a localized lympadenopathy in the draining popliteal lymph nodes. More specifically, FGFR5γ-Fc induced an increase in the total number of cells isolated from the popliteal lymph nodes that was apparent 24 hrs after the protein had been administered and rose to 3.2 times the number of cells isolated from the nodes draining the FGFR2 injection site. The data provided in Fig. 2 demonstrates that subcutaneous administration of FGFR5γ-Fc induced a statistically significant increase in the numbers of B cells (CD19+) and activated B cells (CD19+CD69+) 2 and 3 days after treatment with FGFR5γ-Fc and FGFR2-Fc fusion proteins. Fig. 3 shows that subcutaneous administration of FGFR5γ-Fc induced a statistically significant increase in the frequency of B cells (CD19+) and activated B cells (CD19+CD69+) 2 and 3 days after treatment with the FGFR5γ and FGFR2-Fc fusion proteins. Fig. 4 shows that subcutaneous administration of FGFR5γ-Fc induced a statistically significant increase in the numbers of T cells (CD3+) and activated T cells (CD3+CD69+) 3 days after treatment with the FGFR5γ and FGFR2-Fc fusion proteins. Fig. 5 shows that subcutaneous administration of FGFR5γ-Fc induced a decrease in the frequency of T cells (CD3+) 2 days after treatment and activated T cells (CD3+CD69+) 3 days after treatment with the FGFR5γ and FGFR2-Fc fusion proteins. In Figs. 1-5, the columns marked with an asterisk denote an FGFR5γ-Fc treatment group that differs significantly ($p<0.05$) from the FGFR2-Fc controls as assessed by the students T test.

These experiments demonstrate that FGFR5 induced a localized B cell dominated lymphadenopathy, as shown by an increase in the total number of cells extracted from the lymph node and a preferential increase in both the number and percentage of activated B cells (CD19+CD69+ cells). All of the FGFR5 induced changes were most apparent 3 days after treatment. Although the frequency of T cells declined in the lymph nodes collected from the FGFR5 treated mice, the absolute number of T cells per node increased. These data show that FGFR5 activates the immune system and therefore has the ability to augment responses to antigens in an adjuvant-like manner.

3. An alignment of the murine FGFR5β sequence of SEQ ID NO: 31 with the human FGFR5 sequence of SEQ ID NO: 33 is attached hereto as Exhibit A, with an alignment of the murine FGFR5γ of SEQ ID NO: 32 with the human FGFR5 sequence of SEQ ID NO: 33 being provided in Exhibit B. Based on these alignments, I would expect the polypeptide of SEQ ID NO: 33 to have essentially the same functional properties as the polypeptides of SEQ ID NO: 31 and SEQ ID NO: 32 employed in the above experiments.

4. The undersigned further declares that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 35 of the United States Code.

J. Greg Murison, Ph.D.

21 — November 2003
Date